Research Article

Transcranial direct current stimulation (tDCS) paired with massed practice training to promote adaptive plasticity and motor recovery in chronic incomplete tetraplegia: A pilot study

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Objective: Our goal was to determine if pairing transcranial direct current stimulation (tDCS) with rehabilitation for two weeks could augment adaptive plasticity offered by these residual pathways to elicit longer-lasting improvements in motor function in incomplete spinal cord injury (iSCI).
Design: Longitudinal, randomized, controlled, double-blinded cohort study.
Setting: Cleveland Clinic Foundation, Cleveland, Ohio, USA.
Participants: Eight male subjects with chronic incomplete motor tetraplegia.
Interventions: Massed practice (MP) training with or without tDCS for 2 hrs, 5 times a week.
Outcome Measures: We assessed neurophysiologic and functional outcomes before, after and three months following intervention. Neurophysiologic measures were collected with transcranial magnetic stimulation (TMS). TMS measures included excitability, representational volume, area and distribution of a weaker and stronger muscle motor map. Functional assessments included a manual muscle test (MMT), upper extremity motor score (UEMS), action research arm test (ARAT) and nine hole peg test (NHPT).
Results: We observed that subjects receiving training paired with tDCS had more increased strength of weak proximal (15% vs 10%), wrist (22% vs 10%) and hand (39% vs. 16%) muscles immediately and three months after intervention compared to the sham group. Our observed changes in muscle strength were related to decreases in strong muscle map volume ($r=0.851$), reduced weak muscle excitability ($r=0.808$), a more focused weak muscle motor map ($r=0.675$) and movement of weak muscle motor map ($r=0.935$).

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Conclusion: Overall, our results encourage the establishment of larger clinical trials to confirm the potential benefit of pairing tDCS with training to improve the effectiveness of rehabilitation interventions for individuals with SCI.
Trial Registration: NCT01539109

Keywords: Spinal cord injury, Transcranial magnetic stimulation, Plasticity, Transcranial direct current stimulation, Motor recovery

Introduction

Tetraplegia is the most common (~45%)[1] and disabling form of spinal cord injury (SCI). Individuals with tetraplegia experience extreme disability because their inability to adequately use their torso and upper limbs severely impairs their ability to perform activities of daily living. While many rehabilitation paradigms have been developed to alleviate weakness of the upper limbs, a considerable amount of time is needed to demonstrate measureable improvements.[2–5] For example, in a clinical trial by Hicks *et al.* individuals with tetraplegia achieved significant gains in muscle strength only after receiving a 9 month-long, bi-weekly exercise regimen delivered for up to 2 hrs/day.[2,4,6]

Effectiveness of therapeutic exercise is in part limited because neural structures in the brain and the spinal cord that survive SCI undergo detrimental reorganization that limits their potential to contribute to recovery.[7,8] Briefly, although corticospinal pathways to the upper limbs are spared in a majority of individuals with SCI (>65%),[9,10] a condition commonly referred to as incomplete SCI or iSCI, these pathways become less excitable and slow-conducting compared to pathways devoted to muscles spared rostral to the injury.[11–14] Similarly, although motor cortices in the brain are largely intact after iSCI, cortical maps reorganize in a way that favors the spared (stronger) muscles.[9,15] Specifically, representations of spared (stronger) muscles overtake representations of the less spared (weaker) muscles.[9,15] While therapeutic exercises that retrain weaker muscles hold the potential to reverse these detrimental changes in motor cortices[11,16] and residual pathways,[11] improvements in function remain marginal and are frustratingly slow to be realized[5]. To this end, there has been a recent effort to identify adjunctive approaches that could improve the ability of surviving neural structures to contribute to recovery in patients with iSCI.

One such potential adjunct is noninvasive electrical stimulation delivered to motor cortices and the emergent corticospinal neurons.[17,18] In particular, transcranial direct current stimulation (tDCS) to the motor cortex has begun to arise as a powerful potential adjunct in the field of SCI rehabilitation.[19–23] This is because tDCS has the ability to enhance excitability of motor cortices and corticospinal neurons, and as such facilitate processes of plasticity that ultimately contribute to recovery.[18] For example, in patients with stroke, anodal tDCS delivered to the motor cortex can enhance excitability of the residual motor cortex, restore maps of weaker muscles via up-regulation of N-Methyl D-Aspartate (NMDA) receptor uptake and amplify conduction via spared pathways.[20,24] tDCS can also boost several restorative mechanisms within the spinal cord to facilitate movement of weak muscles below the level of incomplete injury.[25,26] For instance, studies in rats and non-human primates demonstrate that electrical stimulation of the motor cortices can promote sprouting in brainstem-mediated reticulospinal and rubrospinal pathways to enhance weak muscle movement after iSCI.[27,28] In addition, motor cortical stimulation can promote sprouting of spared corticospinal pathways just above the lesion of the spinal cord to propriospinal neurons that project axons below the injury.[26,29] Taken collectively, this evidence has suggested that tDCS delivered to the motor cortex could directly reverse the detrimental reorganization that occurs following SCI by increasing the excitability of motor cortices and corticospinal neurons.

Recent work has begun to build off this evidence and has shown that single short-term sessions of tDCS can transiently improve motor function in subjects with SCI.[20,22,30] However, it remains unclear if long-term application of tDCS concurrently with exercise would promote beneficial plasticity that contributes to recovery in SCI. Therefore here we evaluated whether pairing tDCS with rehabilitation for several sessions could reverse detrimental changes witnessed at the level of the motor cortices and residual pathways and instead augment their plastic potential to contribute to longer-lasting improvements in motor function in iSCI. In a randomized, sham-controlled cohort study, subjects with incomplete tetraplegia either received anodal tDCS paired with rehabilitation or sham tDCS paired with rehabilitation for two weeks. We measured changes in upper limb function using the upper extremity motor score (UEMS), a manual muscle test (MMT), action research arm test (ARAT) and the nine-hole peg test (NHPT). In association, we measured plasticity at the level of the motor cortices and residual pathways using transcranial magnetic stimulation (TMS). Specifically, we recorded changes in motor map output, motor map distribution and corticospinal

excitability. We hypothesized that participants receiving tDCS paired with rehabilitation would show greater restoration of motor cortical maps and excitability of residual pathways to weaker muscles in association with greater recovery of upper limb function than patients receiving sham stimulation with rehabilitation.

Methods (for detailed methods see supplemental file)

Subjects and study design

We enrolled 8 male patients with chronic cervical motor incomplete SCI in a longitudinal, randomized, controlled, double-blinded cohort study design (Fig. 1; NCT01539109) (Table 1). Neurophysiologic and functional outcomes were collected as shown in Fig 1. The exclusion criteria were related to contraindications to TMS as outlined in our previous work[31]. Prior to participating in the study, written informed consent was obtained from all participants, based on the policies of the Institutional Review Board of the Cleveland Clinic and the Department of Defense's Human Research Protection Office.

Rehabilitation: Massed practice (MP) training and transcranial direct current stimulation (tDCS)

All subjects participated in 2 hours of MP training 5 times per week for 2 weeks.[32] Each participant's training program was individualized based on their deficit (Fig. 1**C**). tDCS was delivered concurrently with MP training using 2 electrodes placed in saline-soaked sponges (5×7 cm^2) (Soterix Medical, New York, NY). The anodal electrode was placed over the primary motor cortex (M1) at a site deemed to be devoted to the more affected, weaker muscle lying below the level of the injury. We utilized neuro-navigation to ensure that we had a consistent placement of the anode electrode throughout the intervention period (Fig. 1**D**). The reference (cathodal electrode) was placed over the supra-orbital region, opposite to the targeted M1. For patients in the active tDCS group (tDCS+MP), a current dose of 2 mA was applied concurrently during the first 30 minutes of the first hour of MP training, and again for the first 30 minutes of the second hour of MP training.[33] For patients in the sham tDCS group (sham+MP), the same electrode montage was used, but the stimulator was turned to a 'sham setting'.

Transcranial magnetic stimulation (TMS)

Single-pulse TMS was applied as previously outlined.[31] We recorded TMS-evoked motor potentials (MEPs) in contralateral muscles using surface electromyography (EMG) (PowerLab 4/25 T, AD Instruments Inc., Colorado Springs, CO). Our goal was to use TMS to evaluate if our intervention could reverse the detrimental reorganization that occurs in the brain. Specifically, we sought to understand if representations of a stronger muscle were reduced and representations of a weaker muscle were increased following our intervention. Therefore, we evaluated TMS measures for a weak muscle and a strong muscle on the weaker side of the body (Table 1) in each participant.

All TMS measures were collected in an active state, wherein the target muscle was contracted at 20% of its maximum voluntary contraction. For each muscle, we identified the cortical site eliciting reliable criterion MEPs (at least 200 μV larger than pre-stimulus muscle activity in at least 3/5 trials) at the lowest stimulator intensity, i.e. the 'hot spot'. The intensity of TMS required to elicit criterion-level MEPs at the hot spot was called the active motor threshold (AMT).[34] We then evaluated cortical representational maps for each muscle by delivering five TMS pulses at 110% AMT to sites on a 5×5 grid (10 mm resolution),[35] which was centered on the hot spot for the tested muscle.[36]

Functional tests

Four functional outcome measures were evaluated in our study: upper extremity motor score (UEMS),[37,38] manual muscle test (MMT), nine-hole peg test (NHPT) and action research arm test (ARAT).[39–41] The standard medical research council (MRC) grade scale of 0–5 was used for both the UEMS and MMT, with a 0 representing no detectable motor function and a 5 representing normal motor function. For the MMT, we evaluated the MRC grade in muscles ranging from the shoulder, forearm, wrist, fingers and thumb (*for details see*[31]). For the NHPT, we measured the time required to place and then remove 9 pegs within the board.

Data analysis and statistical testing

We chose to assess TMS metrics that directly related to our hypothesis: corticospinal excitability and motor map changes. We defined corticospinal excitability using AMT. We assessed motor map changes using pre- to post-test changes in motor map volume, the location of the motor map weighted-average center and motor map area. Maps included all 'active' sites within the 5×5 grid. Active sites implied sites that elicited MEPs with peak-to-peak amplitudes more than one standard deviation larger than pre-stimulus activity in ≥ 3 out of 5 trials. We employed a different criterion for mapping to ensure that all possible cortical sites would be included in the motor

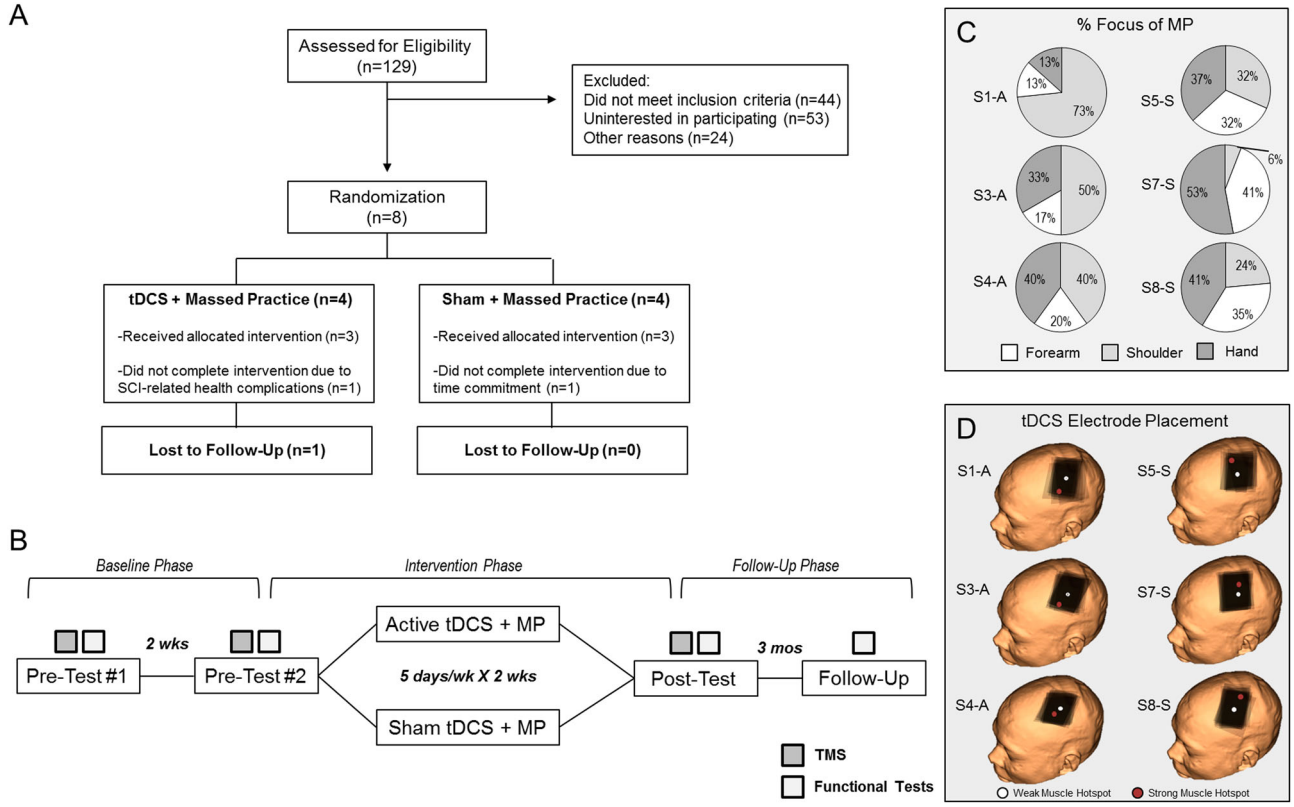

Figure 1 Study design, CONSORT diagram and focus of massed practice training. (A) A CONSORT Diagram of the study demonstrates that 6 of the initial 8 enrolled subjects completed all elements of the study. (B) Subjects completed two baseline testing days (pre-test #1, pre-test #2) that occurred at least 2 weeks apart. Following baseline, subjects participated in massed practice training (MP) with active transcranial direct current stimulation (tDCS) or with sham tDCS. After the intervention, outcome measures were assessed immediately (post-test) and then three months after intervention completion (follow-up). Outcome measures were collected at all time points as indicated by the grey and white boxes. The outcome measures included both neurophysiologic with transcranial magnetic stimulation (TMS) and functional tests. (C) MP was tailored to each subject and focused on muscles within the shoulder, forearm and hand. The percent of exercises that focused on each region is shown for every participant. (D) tDCS anode electrode placement overlaid for all 10 training sessions. Darker areas denote more overlap in electrode placement between the 10 training sessions. The white dots denote the hot spot for the weaker muscle, while the red dots denote the hotspot for the stronger muscle.

map. Motor map volume was defined as the sum of normalized MEPs across all active sites. Area of the map was defined as the total number of active sites in each map.

We assessed changes in clinical impairment and motor function using average MRC grade and the total ARAT score for the weaker upper limb, UEMS score and NHPT. Using these values, we defined longitudinal changes in functional outcome measures as a percent (%) change from pre-test #1.

All statistics were performed in SPSS version 21 (IBM Corp., Armonk, NY). Descriptive statistics for participants within each group (tDCS+MP, sham+MP) were calculated at pre-test #1, pre-test #2, post-test and follow-up. Reliability between measurements at pre-test #1 and pre-test #2 has been reported elsewhere (See Potter-Baker *et al.* for details[31]). The Mann-Whitney U test was used to compare pre- to post-test differences between groups. Significance was defined as $P < 0.05$. For all statistical analysis, we chose to employ non-parametric analyses due to the small sample size of our study. All descriptive statistics are presented as line and bar plots to summarize individual data. Correlation analyses are presented as bivariate Spearman's relationships between changes in outcome measures from pre-test #2 to post-test.[31]

Results

Patient characteristics

The average age of participants in the study was 53.5 ± 4.1 (range 48–62) (Table 1). Six participants completed the clinical study. Participant S2-A withdrew from the study after completing pre-test #1, pre-test #2 and three days of intervention (active tDCS + MP) due to

Table 1 Patient demographics.

Group Assignment	Study Participant	Gender	Age (yr)	AIS Grade	Level of Injury	Duration of Injury (mo)	Etiology	Baseline UEMS (/50)	Weaker Side of the Body	Avg. MRC Grade of Move Impaired Upper Limb	TMS Muscle Pair (Strong/ Weak)	Pain / Spasticity Medications
Active tDCS + Massed Practice Training (tDCS+MP)	S1-A	M	52	D	C2	30	T	31	Right	2.36	FDI / Deltoid	Baclofen, Pregabalin, Celecoxib
	S2-A	M	48	B	C5	98	T	15	Left	1.53	EDC / Tricep	Baclofen pump, Duloxetine
	S3-A	M	52	D	C6	36	T	33	Right	2.30	Bicep / Deltoid	Baclofen, Gabapentin
	S4-A	M	56	D	C4	54	T	40	Right	2.80	Bicep / EDC	Naproxen, Pregabalin, Diclofenac
Sham tDCS + Massed Practice Training (sham+MP)	S5-S	M	54	D	C5	81	T	39	Right	3.24	Deltoid / EDC	Baclofen pump, Gabapentin
	S6-S	M	51	B	C4	372	T	13	Right	2.33	Bicep / Deltoid	Baclofen, Diazepam
	S7-S	M	53	D	C3	182	T	37	Right	2.90	Deltoid / EDC	Baclofen, Diazepam
	S8-S	M	62	D	C3	21	T	42	Right	3.59	EDC / Deltoid	Tramadol, Gabapentin
Avg. ± S.E.M. tDCS+MP			52 ± 1.6			54.5 ± 15.4		29.7 ± 5.3		2.3 ± 0.3		
Avg. ± S.E.M. sham+MP			55 ± 2.4			164 ± 76.8		32.7 ± 6.6		3.0 ± 0.5		

tDCS, transcranial direct current stimulation; UEMS, upper extremity motor score; T, traumatic; MRC, medical research council; MP, massed practice; FDI, first dorsal interosseous; EDC, extensor digitorum communis; SEM, standard error of mean.

complications related to catheter infection. Participant S6-S withdrew from the study after completing pre-test #1 due to schedule constraints. Thus, both AIS B subjects did not complete the study. TMS outcomes for participant S7-S were not available for post-test due to data corruption. MMT and UEMS data for participant S1-A were not available for follow-up because the data was lost.

We observed differences in baseline characteristics between the tDCS+MP and sham+MP group (Table 1). Specifically, we observed that individuals in the sham+MP group were more chronic post-injury (Months Post Injury Average: 164 ± 76.8 mo versus 54.5 ± 15.4 mo) and less impaired (Average MRC Grade of More Impaired Upper Limb: 3.0 ± 0.5 versus 2.3 ± 0.3) in comparison to the tDCS+MP group. Differences in chronicity ($Z = -0.866$, $P = 0.386$) and impairment ($Z = -1.176$, $P = 0.240$) were not found to be significant however. All participants that completed the clinical study were trained with MP for a total of 20 hours (Fig. 1). The focus of MP exercises was dependent on the subject (Fig. 1**C**). Across all participants, the average focus of MP was 37% for the shoulder muscles, 26% for the forearm and 36% for the hand.

Changes in impairment and motor function

Most participants (5 out of 6) demonstrated improvements in their UEMS at post-test (Fig. 2**A**). The net improvement across subjects in the tDCS+MP and sham+MP group for UEMS was comparable at post-test and showed a slight advantage in the tDCS+MP group at follow-up (Fig. 2**A**). Overall, the global patterns of UEMS recovery indicated that myotomes below the level of the subjects' SCI were most dramatically affected by the treatment condition (Fig. 2**D**). For example, subject S1-A (C2 injury) initially had MRC grades below 3 for all myotomes examined in the UEMS. However, after 20 hours of training the same subject substantially improved MRC grades across all myotomes, with a change of MRC grade 2 to 4 in the T1 segment. At this time the subject reported that it was easier to perform tasks of daily living such as turning on a lamp. In another example, subject S5-S (C5 injury) started with an average MRC grade between 2 and 3 for all myotomes in the UEMS. After 20 hours of treatment, the subject improved by at least 1 MRC grade in 4 of the 5 tested myotomes. In tandem with this increase, the subject reported that he had regained the ability to turn on his car and shift the gear stick with his weaker arm. All subjects except S8-S demonstrated improvements in myotomes below the level of injury (Fig. 2**D**).

We also followed changes in the weak muscle MRC grade over the course of the study (Fig. 2**B**). We observed that most participants demonstrated an increase in the MRC grade in their weak muscle, with a slight advantage found in the tDCS+MP group at post-test and follow-up. The majority of participants also demonstrated improvements in the MMT on the weakest side of their body (Fig. 2**C**). Similar to other measures, we observed that the tDCS+MP group had a trending advantage in MMT on the weakest side of the body at both post-test and follow-up (Fig. 2**C**). Notice that this motor recovery advantage in the tDCS+MP group encompassed proximal to distal muscles, and was most pronounced in the shoulder, wrist extensors and thumb (Fig. 2**E**). Thus, the muscles that exhibited the best recovery in the tDCS+MP group in comparison to the sham+MP group were: anterior deltoid (max MRC grade change 2; average change 1 grade), middle deltoid (max 2; average 1), posterior deltoid (max 1; average 1), biceps brachii (max 1; average 1), triceps brachii (max 2; average 1), extensor carpi radialis longus (max 1; average 1), extensor carpi ulnaris (max 2; average 1), extensor digitorum communis (max 1; average 1), first dorsal interosseous (max 2; average 1), opponens (max 1; average 1) and flexor pollsis brevis (max 2; average 1). As previously described, the majority of the motor recovery advantage for the tDCS+MP group occurred below the level of the injury. Further, it is important to note that changes between pre-test #1 and pre-test #2 were minimal for both UEMS and MMT in both groups, thus indicative of an intervention-related effect.

We noted minor changes in functional tests for most participants following the intervention. Improvement in the ARAT was similar between the tDCS+MP and sham+MP group, with only S3-A demonstrating substantial improvements at post-test and follow-up (Fig. 3**A**). Similar observations were noted for the NHPT (Fig. 3**B**). Of note, across MMT, ARAT, NHPT, MMT and UEMS, participant S8-S did not demonstrate any notable improvements (Figs. 2 and 3).

Figure 2 and Figure 3 collectively led to the following conclusions: 1) tDCS+MP provided a trending advantage in improving the strength of muscles across the weaker upper limb at post-test and follow-up, particularly in the shoulder/proximal, wrist extensors and thumb muscles, which were typically the weaker muscles lying below injury; and 2) tDCS+MP had minimal effect on changes in functional tests following the intervention.

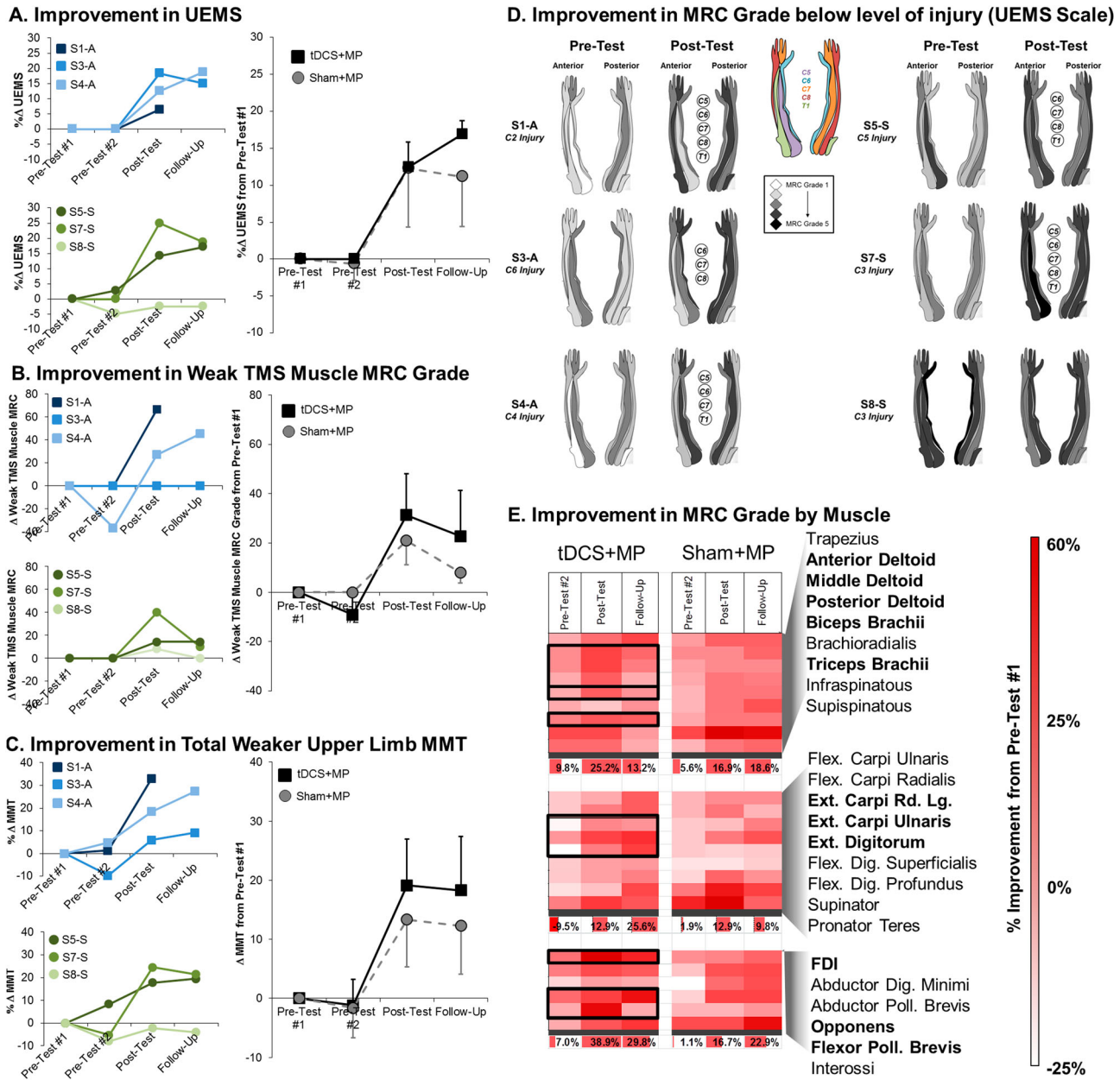

Figure 2 (A) Upper extremity motor score (UEMS) scores following intervention in individuals with chronic incomplete tetraplegia. Most subjects demonstrated an increase in UEMS at post-test and demonstrated sustained or enhanced changes at follow-up. Similar trends were noted across participants for (B) Change in weak TMS muscle medical research council (MRC) grade and (C) change in total MMT for the weaker upper limb. The average change across the participants in each group is denoted in as a black line (tDCS+MP) or gray dashed line (sham+MP). MMT and UEMS was not completed at follow-up for S1-A. (D) Detailed MRC grade changes across the cervical myotomes examined in the UEMS score from pre-test to post-test. Myotomes that demonstrated >15% improvement are denoted in circles at post-test. Most participants demonstrated large improvements in MRC muscle grade for myotomes below the level of injury. (E) Average percent change in MRC grade for 24 muscles across the upper limb in each group. We noted that changes in MRC grade for the anterior deltoid, middle deltoid, posterior deltoid, biceps brachii, triceps brachii, wrist extensors, first dorsal interosseous (FDI), opponens and flexor pollsis brevis were more pronounced in the tDCS+MP group. All values are presented as a percent change from pre-test #1.

Cortical plasticity

We first assessed corticospinal pathway excitability, or AMT, following our intervention. We observed that the weak muscle in the tDCS+MP group had a trending decrease in excitability, as noted by an increased AMT at post-test (Fig. 4). An example of this change is shown in Figure 4, **right**, wherein individuals in the tDCS+MP group had a smaller motor evoked response

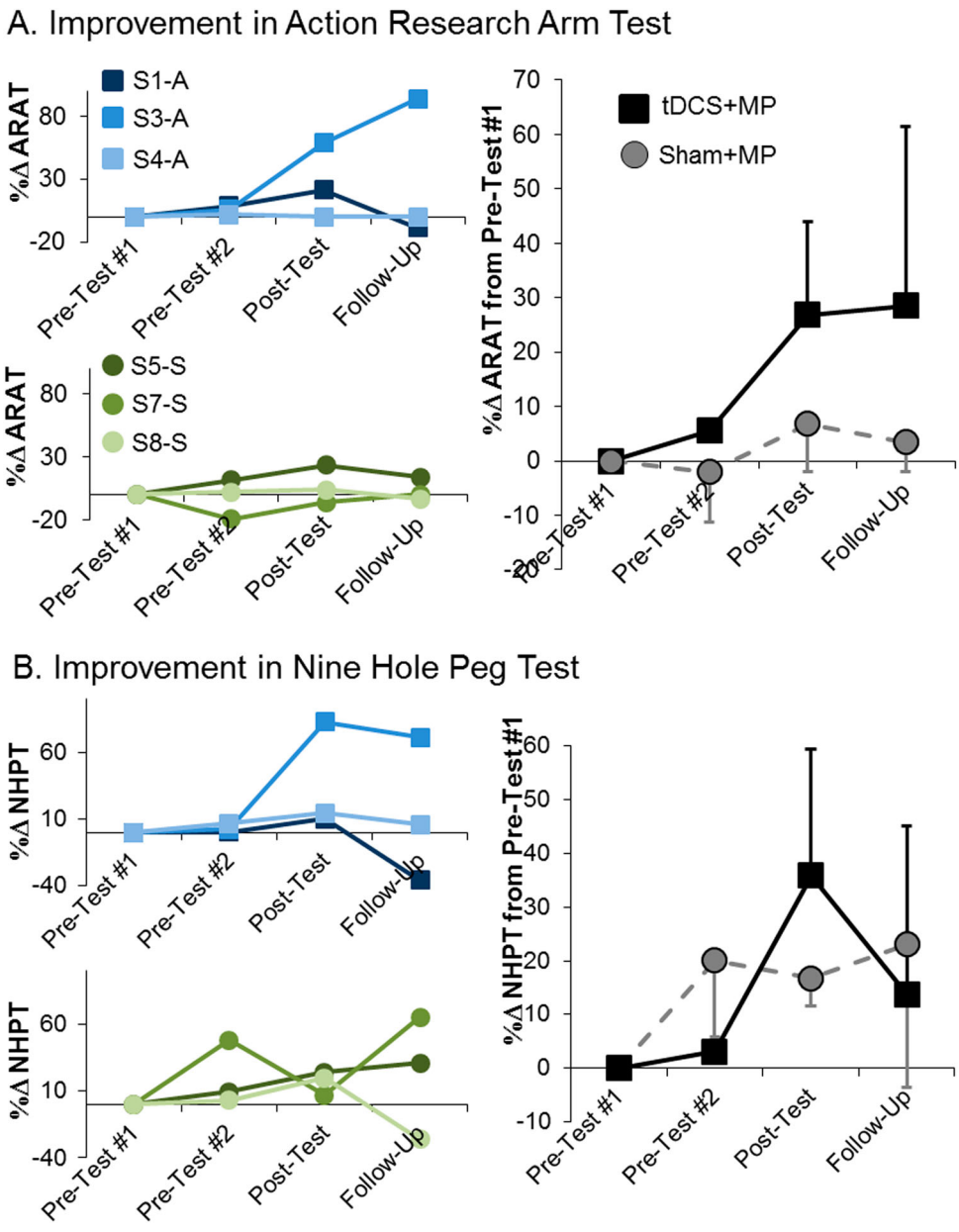

Figure 3 Change in (A) action research arm test (ARAT) and (B) nine hole peg test (NHPT) in individuals with chronic incomplete tetraplegia following intervention. Most subjects demonstrated minimal improvement in functional tasks at post-test and follow-up. S3-A demonstrated the most improvement in ARAT and NHPT. Values are presented as a percent change from pre-test #1. The average change across the participants in each group is denoted in as a black line (tDCS+MP) or gray dashed line (sham+MP).

at the same intensity at post-test in comparison to sham+MP. A preliminary analysis suggested that participants who had the most improvement in UEMS after the intervention were those with a less excitable weak muscle (high AMT; r=0.808) (Fig. 4, inset).

We found that changes in the volume and area of the weaker muscle motor maps at post-test were minimal or variable in the tDCS+MP and sham+MP groups. Changes from pre-test #2 to post-test for the tDCS+MP group were $-111\ mm^3$, $-388\ mm^3$ and $-42\ mm^3$, while those in the sham+MP group were $+802\ mm^3$ and $-405\ mm^3$, respectively.

In contrast, the representation volume and area of the stronger muscle was substantially changed at post-test for both the tDCS+MP and sham+MP group. In the tDCS+MP group, strong muscle map volume was markedly reduced but in the sham+MP group the strong muscle motor map volume increased at post-test (Fig. 5**A**). Of note, subjects S3-A and S4-A demonstrated a reduction of ~50% in strong muscle map

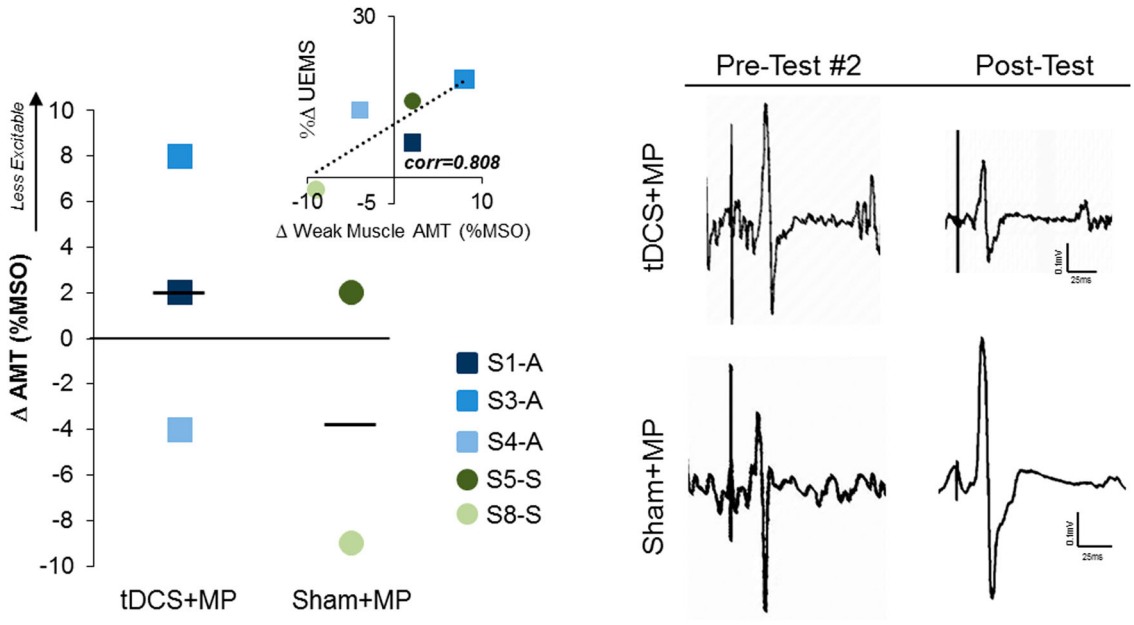

Figure 4 Change in weak muscle excitability, or active motor threshold (AMT), in the tDCS+MP group and Sham+MP group. (Left) Individuals in the tDCS+MP group had a reduced excitability in their weaker muscles, as noted by an increase in AMT at post-test in comparison to Sham+MP. The average of each group is shown as a straight line symbol. (Inset) We noted that a reduction in excitability in the weaker muscle from pre-test #2 to post-test was related to more improvements in UEMS at post-test. (Right) Example motor evoked potentials at pre-test #2 and post-test demonstrating the reduction in excitability at post-test for the weak muscle in the tDCS+MP group.

volume at post-test. In comparison, S8-S demonstrated a ~200% increase in strong muscle map volume, while S5-S demonstrated minimal changes in strong muscle volume at post-test. Our observed changes in volume translated directly to changes in muscle map area. Overall, we found that the area for the stronger muscle decreased by up to 10 sites for the tDCS+MP group (average decrease of 4 sites) and increased by up to 8 sites for the sham+MP group (average increase of 5 sites) at post-test. Note that our observed changes in strong muscle motor map volume were related to UEMS improvement at post-test ($r=0.82$), wherein individuals that had a decrease in the volume of the representation of their strong muscle demonstrated the most improvement in UEMS at post-test (Fig. 5**A**).

We also evaluated the motor map distribution by determining the number of map sites at which the MEP amplitude exceeded 25%, 50% and 75% of the maximum MEP (M-MEP) elicited in the map for each muscle (Fig. 5**B**). The goal of this additional analysis was to determine whether the cortical map representations were more focused or diffuse following intervention. In general, we found that the distribution of MEP amplitudes did not substantially change in the strong muscle at post-test (data not shown). In contrast, we did note a change in MEP distribution in the weak muscle representation at post-test between groups. In the tDCS+MP group, we observed that the weak muscle motor map had become more focused. As displayed in Fig. 5**B**, the average percentage of map sites that demonstrated MEPs >75% the maximum MEP (M-MEP) changed from 17% to 29% between pre-test #2 and post-test for the tDCS+MP group. Taken together, this indicated that although less motor map sites were actively eliciting MEPs in the tDCS+MP group, sites that were excitable were more robust and focalized. Notice also that participants demonstrating such a more focused weak map muscle representation at post-test were also those with the most improvements in UEMS at post-test ($r=0.675$).

In comparison, individuals in the sham+MP group demonstrated a more diffuse motor map at post-test (Fig. 5**B**). Specifically, note that the average percentage of map sites demonstrating MEPs between 50 and 75% M-MEP changed from 16% to 40% between pre-test #2 and post-test for the sham+MP group. Thus, weaker motor maps in the sham+MP group saturated more cortical sites and demonstrated smaller MEPs.

As a final measure of plasticity, we also evaluated the change in the weighted center of gravity (CoG) of the weak muscle map between pre-test #2 and post-test in both groups (Fig. 5**C**). In general, we noted variable movement in the CoG of motor maps

in all subjects. Overall we found that subjects who demonstrated plasticity more medially were also those with the most improvement in UEMS at post-test (Fig. 5**C**). For example, in subject S3-A, the deltoid moved medially by 10 mm and demonstrated an increase in UEMS ~20%. In contrast, subject S8-S, the deltoid muscle in S8-S moved laterally by 15 mm and the subject showed a decrease in UEMS by ~5%.

Discussion

The main goal of the present study was to determine whether pairing tDCS with rehabilitation for several sessions could potentially reverse detrimental changes and instead augment the potential for adaptive plasticity offered by motor cortices and residual pathways to elicit longer-lasting improvements in motor function in iSCI. In addition, the present study served as a pilot experiment to help derive variance of treatment effect and estimate sample sizes for performing a larger study in the future. The results of our study therefore are preliminary and seem to suggest that pairing tDCS with two weeks of massed practice training could help improve and maintain the strength of weak proximal and hand muscles in individuals with incomplete tetraplegia in association with adaptive neurophysiological changes. Based on our observations, we encourage the establishment of larger scale studies to determine whether tDCS can indeed result in significant clinical functional gain in association with representation plasticity changes in the weak and stronger muscles.

Recent studies that have paired tDCS with exercise for a single session in individuals with iSCI have suggested that tDCS+exercise may have a potential functional advantage for improving motor recovery.[22,30,42] Building on this work, our pilot study observations indicate that long-term application of tDCS in tandem with exercise could improve the MRC grade in muscles across the weaker upper limb (Fig. 2), including those assessed during UEMS (Fig. 2**D**). Our pilot findings also indicate that tDCS+MP could result in a more recovery in the muscle grade of muscles innervated below the level of injury (Fig. 2**D, E**). In our cohort, improvements in UEMS at post-test occurred in 100% of individuals in the tDCS+MP group but only 66% of individuals in the sham+MP group. Further, regardless of group, MRC grades were maintained at 3 months after the end of the intervention, with a slight advantage in the tDCS+MP at follow-up (Fig. 2**A, B, E**). This observation has important implications for implementation of tDCS+MP in a clinical setting in chronic iSCI. All the individuals in this study were in the chronic phase of injury (>20 month post-SCI) and therefore were considered beyond the period which spontaneous recovery is expected.[43] Indeed, we did not note any significant changes in motor function between pre-test #1 and pre-test #2 in the present report.[31] Our observations from this pilot study thus suggest that it is possible to achieve additional functional gains in the chronic stages of injury, as was previously suggested[44]. Further, since improvements in MMT and UEMS were maintained at follow-up in most of our subjects (Fig. 2**B, E**), our results suggest that tDCS+MP could offer a slight advantage for improvements in MMT and UEMS even if used for a short treatment window (2 weeks).

While our observations in the present pilot study suggest a potential positive effect of tDCS+MP in comparison to sham+MP for changes in muscle MRC grade, we cannot discount that subjects in both groups demonstrated minimal or variable improvements in functional outcome measures, such as ARAT and NHPT (Fig. 3). We attribute this observation to the fact that the ARAT and NHPT required muscles that were not heavily focused on during MP across subjects: distal hand muscles. Specifically, we focused the majority of training exercises on each subject's weaker TMS muscle group in the upper limb (Fig. 1). As shown in Table 1, the majority of weaker muscles in our subjects were proximally located. Further, as indicated by Figure 1**C**, in most subjects, MP training focused on the hand was <40%. As a result, since our exercise training targeted proximally located muscles it is understandable why we observed larger improvements in MMT of proximal muscles in comparison to grasp/hand dexterity (Fig. 2, 3). Further, as previously mentioned, a minimum of 9 months is required to demonstrate significant functional outcome measures in subjects with SCI.[2] Thus, it is also possible that our paradigm may have been too short to observe meaningful changes in ARAT and NHPT.

Maladaptive changes in the brain and spared pathways have been implicated to hinder motor recovery following SCI.[45] Here, we observed that tDCS as an adjunct to therapeutic exercises can alter excitability, representational area, volume and distribution of weak and strong muscles in the motor cortex and that such plasticity is correlated with recovery (Fig. 4, 5). While we anticipated the majority of tDCS-mediated plasticity changes to occur in the weaker muscles below the level of injury, our observations suggest the opposite. Specifically, our pilot study suggests that the area and volume of the weaker muscle cortical representations did not change over the course of the treatment for the

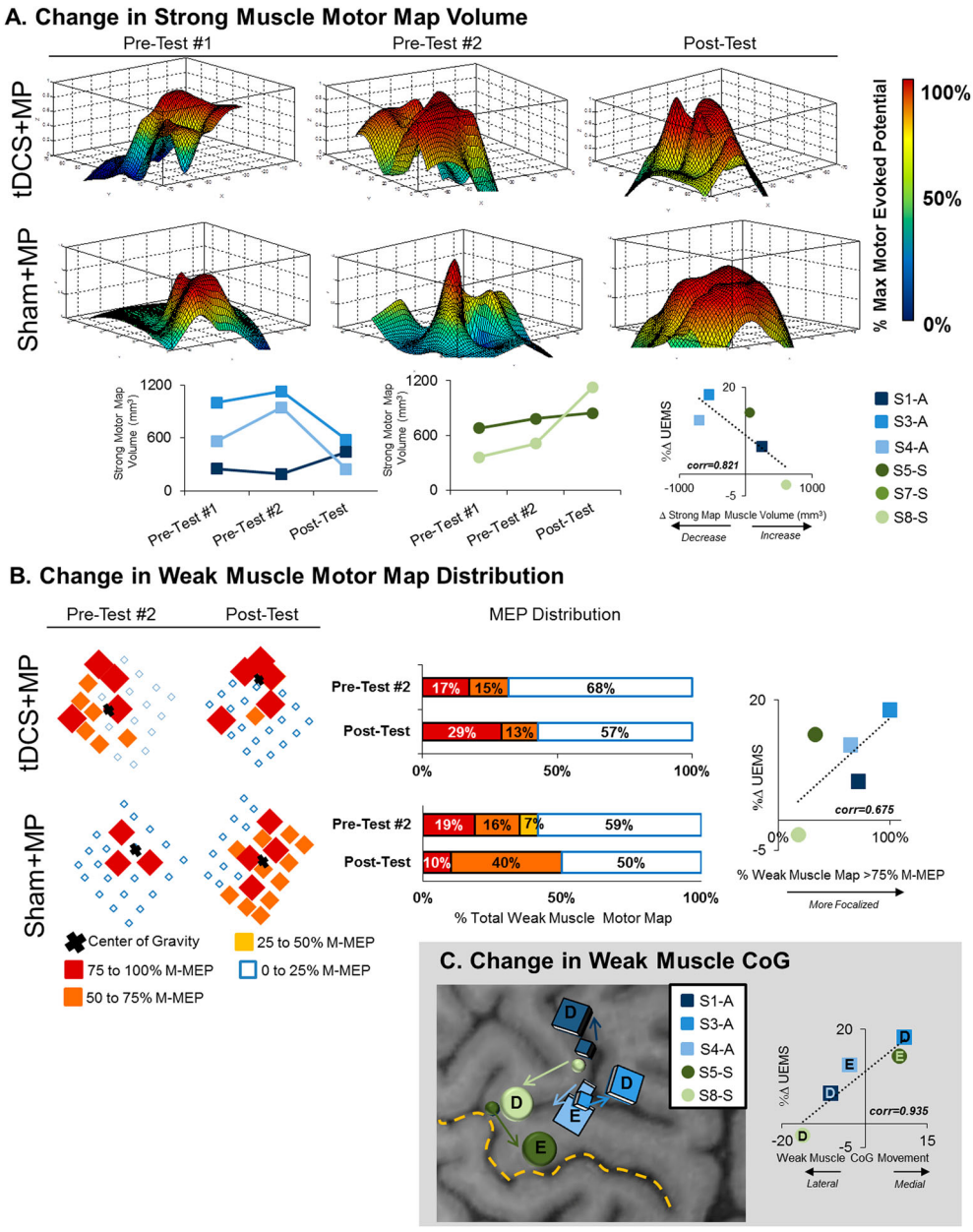

Figure 5 (A) Change in volume for the representation of the stronger muscle in the tDCS+MP group and Sham+MP group. (A, upper) Example of volume changes of the stronger muscle in S3-A at pre-test #1, pre-test #2 and post-test. Example of volume changes of the stronger muscle in S8-S at pre-test #1, pre-test #2 and post-test. Example motor maps are shown as a 3-D contour plot normalized to the MEP_{Maxima} for each respective map. Overall we noted that subjects in the tDCS+MP group showed a reduction in the volume of their strong muscle motor map at post-test in comparison to the sham+MP group. Participants with more decreases in the volume of their stronger muscle representation also were found to demonstrate more improvement in UEMS immediately after intervention. (B) We found that the tDCS+MP group also demonstrated a more focused representation of the weaker muscle following intervention than the sham+MP group. An example distribution change is shown for the representation of the weaker muscle in the tDCS+MP group and Sham+MP group. MEP amplitudes in distribution maps are presented as a $\%MEP_{Maxima}$ (M-MEP), wherein red denotes sites that were $\geq$75% MEP_{Maxima}, orange denotes sites that were $\leq$75% and $\geq$50% MEP_{Maxima}, yellow denotes sites that were $\leq$50% and $\geq$25% MEP_{Maxima} and blue represents sites that were $\leq$25% MEP_{Maxima}. Individuals with a more focused weak muscle map, as denoted by a higher number of map sites demonstrating MEPs >75% the maximum MEP (M-MEP) had more improvement with UEMS at post-test. (C) Movement of the center of gravity (CoG) for the representation of the weaker muscle muscle for individuals with incomplete tetraplegia following intervention. Arrows denote movement of the CoG from pre-test #2 to post-test for each individual muscle. Yellow dashed line denotes the central sulcus in anatomical map. Here, E represents the extensor digitorum communis and D denotes the middle deltoid. Participants with more medial movement of their weaker muscle representation (center of gravity; CoG) demonstrated more gains in motor function at post-test for the UEMS.

tDCS+MP and sham+MP groups. Instead, most participants in the tDCS+MP group (2 out of 3) demonstrated decreases in the volume and area of the representation of their strong muscle, while the whole sham+MP group displayed the opposite behavior (Fig. 5**A**). While our plasticity findings should be interpreted with caution given the small sample size, we believe that our observations may be the result of both tDCS driving adaptive plasticity changes, as has been previously shown in stroke,[46] and the length of the intervention. Specifically, we hypothesize that the duration of two weeks was sufficient to drive adaptive changes in the strong muscle for the tDCS+MP group (i.e. reduction of volume), but due to presumed increased damage to corticospinal pathways innervating the weak muscle, more time would have been needed to illustrate plasticity in weak muscle volume. Although, our observations did suggest that the weak muscle representation became more focused in the tDCS+MP group (Fig. 5**B**). Thus, despite a short intervention window, small plasticity changes within the weaker muscle still were observed, and found to be related with recovery across subjects (Fig. 5). In contrast, individuals in the sham+MP group demonstrated weaker patterns of plasticity. Specifically, our results suggest that individuals in the sham+MP group were still trying to rely on recruitment of the weaker muscle as noted by a peripheral spread in the activation of the weak muscle motor map (Fig. 5**B**) and that such plasticity was not related to recovery. As a result, future studies will need to confirm that two weeks of tDCS paired with exercises can alleviate detrimental changes of strong muscle cortical representations while improving adaptive plasticity of weak muscle representations to ultimately drive functional recovery (Fig. 5).

The role of cortical motor representation movement can also not be overlooked (Fig. 5**C**). Due to the variable nature of our subjects' injury, different weak muscles were chosen across patients (Table 1). We believe that this is why similar movement patterns within a group were not demonstrated. However, regardless of muscle, we found that subjects demonstrating a medial movement in their weak muscle representation (Fig. 5**C**) had a greater recovery of UEMS. This observation is not surprising given that multiple studies have suggested that cortical map shifts toward the supplementary motor area (SMA) and dorsal premotor area is common after strength training.[47–49]

As a means to add rigor to our study design, we employed the use of a baseline control phase (pre-test #1 and pre-test #2). Our previous work has shown that outcome measures can be variable in subjects with SCI.[31] Indeed, in the present study we observed some variability in TMS metrics for cortical plasticity from pre-test #1 to pre-test #2 among our small cohort (Figs. 4, 5). Therefore it was important for us to also evaluate whether our witnessed changes in function and neurophysiology during the intervention phase were confounded by variability issues. To do this, we first performed a correlation analysis to determine if changes in TMS metrics during the baseline control phase (between pre-test #1 and pre-test #2) were related to changes in functional outcomes during the baseline control phase. We assessed possible relationships between changes in excitability, motor map volume, distribution, CoG movement and changes in UEMS, MMT, NHPT, and ARAT. However, we noted no direct correlation between changes between baseline changes in motor maps (pre-test #2 minus pre-test #1) and baseline changes in function ($R^2 < 0.1$). Further, we also observed that the magnitude of changes witnessed during the intervention phase was more substantial than the baseline control phase. For example, strong muscle motor map volume changed by 100 mm^3 between pre-test #1 and #2 for S3-A, but changed by approximately 600 mm^3 from pre-test #2 to post-test. In addition, it should be noted that a baseline control phase has rarely been used in SCI clinical study and therefore its addition has added tremendous rigor to this small study. Thus, inclusion of a baseline control phase generates greater confidence that effects witnessed following interventions were less likely to be related to natural shifts in functional performance.

While our findings were consistent across most participants, we noted that one subject demonstrated abnormalities worth mention. Mainly, we observed little to no change in improvement for S8-S over the course of the study. We propose that a lack of benefit could be attributed to the level of impairment. As shown in Table 1, S8-S demonstrated the highest amount of remaining motor function (UEMS score = 42) across subjects, with a majority of his muscles demonstrating ≥ 4 MRC grade (Fig. 2). Therefore, it is possible that the lack of recovery in S8-S was the result of a ceiling effect.

Although the results of this cohort study are promising, a number of questions and limitations arise that should be addressed in future studies. First, as the first study to evaluate whether long-term pairing of tDCS with training of weaker muscles would serve as an ideal synergistic paradigm to promote adaptive plasticity, we opted to study plasticity in subject-specific muscles. As a result, different muscle pairings were chosen across participants (Table 1) thereby limiting

data analysis. Second, as previously stated, we acknowledge that our measurements of plasticity (motor maps) were variable and different across subjects from pre-test #1 to pre-test #2. However, we noted no direct correlation between changes between baseline changes in motor maps (pre-test #2 minus pre-test #1) and baseline changes in function. Further, the magnitude of changes observed at the end of intervention was greater than those found during baseline. Thus, we have more confidence in our observed relationships between plasticity and motor function after the intervention (post-test minus pre-test #2). Third, we acknowledge that subjects in our sham group were inherently less impaired, although there were no significant differences between groups, and that this may have influenced our observed benefits with tDCS. Fourth, we also cannot discount that the effects of tDCS are highly variable.[50] Thus, even though we did include several methodological controls to ensure more reliable tDCS application, including neuronavigation, a 2 mA current[51] and subsequent tDCS application,[33] it is still possible that variability of tDCS affected our observations. Fifth, all our participants were on medications for pain or spasticity that are known to affect excitability of cortical and spinal circuits. For example, baclofen and gabapentin have been shown to decrease intracortical facilitation, thereby reducing excitability.[52,53] As a result, our findings may have been affected by the various medications taken by each subject. Sixth, we also cannot discount the effect of metaplasticity on our experimental design. For example, in stroke, tDCS applied *before* rehabilitation has shown to be more advantageous.[54] Thus, it is possible that tDCS may not have provided any advantages during therapy. However, since we continued rehabilitation after tDCS was turned off, we hypothesize that our intervention design still may have resulted in improved functional benefit. And finally, as a cohort study, our analysis included a small number of subjects. Therefore, future studies using a larger cohort would be needed to confirm our findings. In fact, because of our observations in the change of MRC grade of the weaker muscles in the present pilot study, we have determined that a sample size of 28 subjects (14 per group) will be needed to achieve 80% power in the future larger study.

Conclusion

In summary, our pilot study observations suggest that pairing tDCS with training of weaker muscles for two weeks may provide some advantage in improving strength in proximal/hand muscles below the level of injury as well as a change in the size, excitability and location of motor cortical representations of weak and stronger muscles in subjects with SCI. We observed that such plastic changes are related to improvements to the UEMS directly after the intervention. Our results outline the need for larger clinical trials to confirm the potential benefit of pairing tDCS with training to improve the effectiveness of current rehabilitation interventions for individuals with SCI. Further, it is important that future work understand whether there are individuals who would respond best to tDCS, or other types of neuromodulation.

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Author Disclosure Statement

No competing financial interests exist.

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